

Brief Communication (Original)

Detection of *Neisseria gonorrhoeae* in Palestinian women using polymerase chain reaction

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Background: *Neisseria gonorrhoeae* is an exclusive human pathogen that primarily infects the urogenital epithelia. Infections caused by *N. gonorrhoeae* are considered the second major cause of sexually transmitted disease after *Chlamydiae* worldwide. Although the urethra and the uterine cervix serve as the initial sites for gonococcal infections in men and women, infection of the conjunctiva, pharynx, tendons, joints, as well as rectal mucosa are also reported.

Objectives: The objectives of this study were to introduce molecular techniques such as polymerase chain reaction (PCR) to detect *N. gonorrhoeae* directly from endocervical swabs. In addition, it provides a picture of *Neisseria gonorrhoea* infection among a sample of Palestinian women in West Bank.

Methods: Two hundred and thirteen endocervical swabs were collected from sexually active married women with endocervical abnormalities attending healthcare clinics. DNA was extracted directly from the swabs and PCR was performed using specific primers targeting the *orf1* region of the genome.

Results: The results obtained indicated that the percentage of positive cases of *N. gonorrhoeae* among the women tested was 1.40%.

Conclusion: Implementing guidelines for comprehensive screening of men and women with more sensitive tests may improve detection and management of sexually transmitted infections.

Keywords: Endocervix, *Neisseria gonorrhoeae*, Palestine, PCR, sexually transmitted disease

Gonorrhea is one of the most prevalent sexually transmitted diseases (STD) worldwide [1]. The etiological agent of gonorrhea is *Neisseria gonorrhoeae*. According to World Health Organization (WHO) estimates, the global incidence of gonorrhoeae is approximately 60 million cases per year [2]. According to CDC estimates, the incidence of gonorrhoeae in the USA is about 700,000 cases per year [3].

Urogenital disease due to gonorrheal infection most often occurs as asymptomatic (50%) or mild urethritis and cervicitis. It can also produce serious sequelae, including endometritis and salpingitis in women, resulting in spontaneous abortion, premature

delivery, and ectopic pregnancy [4, 5]. The presence of symptoms is dependent on the site of infection. In females, the infection is accompanied by purulent (or pus-like) discharge, which may be foul or bad smelling, inflammation, redness, swelling, dysuria, and a burning sensation during urination. Infections with *N. gonorrhoeae* could facilitate HIV infection especially in the young adults. The treatment of STDs reduces the transmission of HIV by more than 40-60% [6-8].

Transmission of *N. gonorrhoeae* to neonates usually occurs during delivery as they pass through the infected birth canal. Unsafe sexual behavior increases the risk of contracting gonorrhea while the use of condoms reduces transmission [9]. There is no known immunity to gonorrhea and no vaccine. All sexually active persons in a population are susceptible to the infection.

Gonorrhea is the second most common STD worldwide. In the Middle East, few studies have been carried out to determine the incidence or prevalence rate of gonorrhea. In Saudi Arabia, the incidence of STDs from 1995 to 1999 ranged from 936 to about 1747 cases per 100,000 populations [10].

In Palestine, the prevalence of STDs has not been determined. This study was conducted in cooperation with the Governmental Primary Health Care Division in the West Bank, Palestine. Genital infection caused by *N. gonorrhoeae* in females is known to contribute to dissemination of gonorrhea in the community. The objective of this study was to provide information regarding the prevalence of *N. gonorrhoeae* infection among Palestinian women suffering from endocervical abnormalities. We introduced specific and sensitive molecular methods not used before, such as polymerase chain reaction (PCR), so that such methods might be used by the Ministry of Health of Palestine for comprehensive screening of men and women for gonorrhea as well as other STDs.

Materials and methods

Study samples

Two hundred thirteen endocervical swabs were randomly collected from sexually active women, 16-25 years old, attending primary health care clinics in Ramallah area in the West Bank of Palestine, and suffering from endocervical abnormalities. Specimens were collected by the attending physicians. Single and pregnant women were excluded.

Specimen collection and processing

Two sterile wooden cotton swabs were used to collect the endocervical specimens from each woman. The first cotton swab was used to remove excess mucus from the endocervical area while the second swab was inserted into the endocervical canal, rotated 360 degree in one direction carefully withdrawn to avoid contamination, placed in the transport media (Sterile Amies Clear, Biomed) and stored at -20°C to be used for DNA extraction.

DNA extraction

DNA was extracted as described by Santos et al. 2003 [11]. Briefly, endocervical swabs were put in eppendorf tubes and centrifuged at 8000 rpm for two minutes. Triton (4 µL) and proteinase K (4 µL, from 10 mg/mL stock) were added to each tube and the mixture incubated at 55°C for 90 minutes.

Proteinase K was inactivated by placing the tubes at 95°C for 30 minutes. Then, the samples were centrifuged and the supernatant containing DNA was stored at -20°C for subsequent PCR amplification.

Polymerase chain reaction

For PCR, a set of primers were used to target the *orf1* gene to amplify a 260 bp product specific for *N. gonorrhoeae*. The sequence for the forward primer Ngu1, 5'-CAA CTA TTC CCG ATT GCG A-3' targeting the region from nucleotide 221 to 239, and the reverse primer Ngu2, 5'-GTT ATA CAG CTT CGC CTG AA-3' targeting nucleotide 461 to 480 was used. The PCR reaction was performed in 100 µL reaction volumes containing 50 pmol of each oligonucleotide primer, ready mix (in-vitrogen) and 50 ng of genomic DNA. For each PCR amplification, a positive control, a negative control and DNA ladder (Promega) was used.

Thermal cycling for *N. gonorrhoeae* included an initial denaturation step at 94°C for five minutes followed by 40 cycles. Each cycle consisted of 94°C for 30 seconds, 52°C for 30 seconds and 72°C for one minute with the final extension step at 72°C for 10 minutes [12]. The PCR product was run on 2% agarose gel in 1x TAE buffer containing 0.5 µg/mL ethidium bromide. Samples were electrophoresed at 5-8 V/cm. The size of the DNA amplicons was determined by comparing them with a 1kbp DNA ladder included in each gel. Samples were visualized under UV and photographed using a Polaroid camera.

Analysis of PCR products

Seven and a half microliter (7.5 µL) of each PCR reaction was mixed with 2 µL of DNA gel loading dye and run on a 2% agarose gel in 1x TAE buffer containing 0.5 µg/mL ethidium bromide. Samples were electrophoresed at 5-8 V/cm. The size of the DNA amplicons was determined by comparing them with a 1kbp DNA ladder included with each gel. Samples were visualized under UV and photographed using a Polaroid camera (Fotodyne, UK).

Ethical consideration

This study was conducted in cooperation with the Governmental Primary Health Care Division in the West Bank, Palestine. Informed consent according to WHO recommendations for STDs was obtained from all women participating in this study and the privacy of the human subjects was protected.

Results

Endocervical swabs from 213 women attending primary health care clinics in the West bank, Palestine were collected. DNA was extracted from the endocervical swabs and PCR was performed using primers targeting the *orf1* gene specific for *N. gonorrhoeae*.

The percentage of positive cases of *N. gonorrhoeae* infections was found to be 1.40%. A representative photograph of the results obtained by PCR is shown in **Fig. 1**

Discussion

Gonorrhea is a major sexually transmitted infection and the second most common STD worldwide. The rising number of cases, the emergence of antibiotic resistance [3], and the increasing risk of sexual transmission of HIV [3, 4], emphasizes the concern by major health organizations regarding *N. gonorrhoeae*.

In the Middle East, data for the incidence and prevalence of gonorrhea are either estimated or unavailable. In Palestine, there is no available literature regarding the incidence of gonorrhea. Only few studies have been carried out on other sexually transmitted pathogens such as *Trichomonas vaginalis* [13]. This can be due to the reliance of laboratories in these countries on conventional methods such as culture and Gram stain to detect *N. gonorrhoeae*. False-

negative results can be attributed to the fastidious nature of this organism, delays in specimen transport and processing, storage, and inhibition of growth by certain components of the selective media used [14].

To obtain reliable and accurate data regarding prevalence and incidence of gonorrhoeae, it is important to utilize nucleic based methods such as PCR, which has high sensitivity and specificity. In addition, it provides an alternative method for screening both symptomatic and asymptomatic individuals for *N. gonorrhoeae* [12, 14].

In this study, the first conducted in this country, we used PCR to detect *N. gonorrhoeae* directly from endocervical swabs. The prevalence among our study samples was found to be 1.4%. This result is an agreement with studies conducted in Israel [15]. Although the results obtained are in agreement with other Middle Eastern countries such as Saudi Arabia and Israel, it is much higher than rates obtained in industrialized countries in Europe and North America.

In conclusion, control of gonorrhea is not a simple task. Due to the fastidious nature of this organism, it is extremely important to implement highly sensitive and specific molecular methods for the detection of this organism. This can contribute to enhanced diagnosis, proper treatment, and surveillance of antimicrobial resistance.

The authors have no conflict of interest to report.

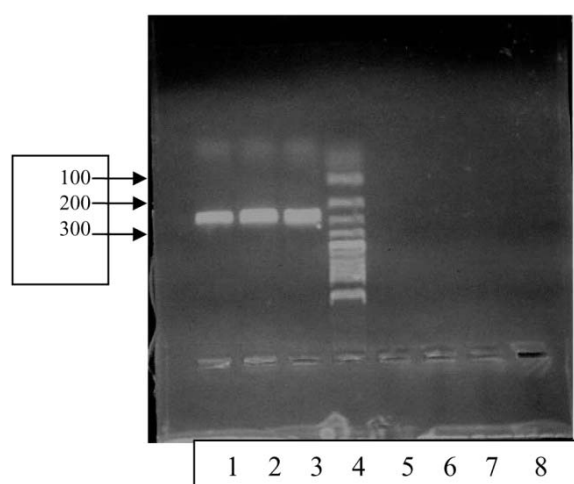


Fig. 1 Representative agarose gel showing the PCR products for the *orf1* gene. Lane 1: positive control for *Neisseria gonorrhoeae*, Lane 2: positive sample, Lane 3: positive sample, Lane 4: 100 bp ladder, Lane 5 and 6: negative samples, Lane 7: negative control, Lane 8: blank.

References

1. Mandell G, Bennett J, Dolin R. Principles and Practice of Infectious Diseases. 6th ed. New York: Churchill Livingstone; 2004.
2. Tapsall JW. What management is there for gonorrhea in the postquinolone era? *Sex Transm Dis.* 2006; 33: 8-10.
3. Kenneth Todar. *Todar's Online Textbook of Microbiology.* University of Wisconsin-Madison Department of Bacteriology; 2008.
4. Maria R, Johanne S, Manonmany V, Mafizur R, Elise K, Per H. Chlamydia and gonorrhea in pregnant Batswana women: time to discard the syndromic approach? *BMC Infect Dis.* 2007; 7:27.
5. Wasserheit J, Holmes K. Reproductive tract infections: challenges for international health policy, programs, and research. In: Germain A, Holmes K, Piot P, Wasserheit J, eds. *Reproductive tract infections: global impact and priorities for women's reproductive health.* New York: Plenum Press; 1992. p. 10-13.
6. Chalamilla G, Mbwana J, Mhalu F, Mmari E, Majigo M, Swai A, et al. Patterns of sexually transmitted infections in adolescents and youth in Dar el Salaam, Tanzania. *BMC Infect Dis.* 2006; 6:22.
7. Brabin L, Kemp J, Obunge O, Ikimalo J, Dollimore N, Odu N et al. Reproductive tract infections and abortion among adolescents in Nigeria. *Lancet* 1995; 345:3000-4.
8. Mwakagile D, Mmari E, Makwaya C, Mbwana J, Biberfeld G, Mhalu F, et al. Sexual behavior among youths at high risk for HIV-1 infection in Dar es Salaam, Tanzania. *Sex Transm Infect.* 2001; 77:255-9.
9. Washington W, Koneman E, Allen S, Procop G, Schreckenberger P, Woods G, et al. *Konemans Color Atlas and Textbook of Diagnostic Microbiology.* 6th ed. Philadelphia: Lippincott Williams & Wilkins; 2005.
10. Madani T. Sexually transmitted infections in Saudi Arabia. *BMC Infect Dis.* 2006; 6:3.
11. Santos C, Texeira F, Vicente A, Astoifi-Filho S. Detection of *C. trachomatis* in endocervical smears of sexually active women in Manaus-AM, Brazil, by PCR. *Braz J Infect Dis, Brazil.* 2003; 7:91-5.
12. Cotchfelt K, Welsh L, DeBonville D, Rosenstraus M, Quinn T. Detection of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* in genitourinary specimens from men and women by a coamplification PCR assay. *J Clin Microbiol.* 1997; 35:1536-40.
13. Al-Hindi A, Lubbad A. *Trichomonas vaginalis* infection among Palestinian women: prevalence and trends during 2000-2006, *Turk J Med Sci.* 2006; 36: 371-5.
14. Chaudhry U, Ray K, Bala M, Saluja D. Multiplex polymerase chain reaction assay for the detection of *Neisseria gonorrhoeae* in urogenital specimens. *Curr Sci.* 2002; 83:634-40.
15. Bamberger E, Siegler E, Markler-Shiron E, Petel M, Steinberg J, Gershtein R, et al. *Chlamydia trachomatis* infections in female soldiers. *Israel Emerg Infect Dis.* 2003; 9:1344-13.